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Journal of Environmental Science and Health, Part B
Publication details, including instructions for authors and subscription information:
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George F. Antonious [a]; Tejinder S. Kochhar [b]; Robert L. Jarret [c]; John C. Snyder [d]
[a] Department of Plant and Soil Science, Kentucky State University, Frankfort, KY [b] Department of Biology, Kentucky State University, Frankfort, KY [c] U.S. Department of Agriculture/Agriculture Research & Service, USDA/ARS Plant Genetic Resources Conservation Unit, Griffin, GA [d] Department of Horticulture, University of Kentucky, Lexington, KY, USA

Online Publication Date: 01 October 2006

To cite this Article Antonious, George F., Kochhar, Tejinder S., Jarret, Robert L. and Snyder, John C.(2006)'Antioxidants in Hot Pepper: Variation Among Accessions',Journal of Environmental Science and Health, Part B,41:7,1237 — 1243
To link to this Article: DOI: 10.1080/03601230600857114
URL: http://dx.doi.org/10.1080/03601230600857114

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Journal of Environmental Science and Health Part B, 41:1237–1243, 2006
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ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601230600857114

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Antioxidants in Hot Pepper: Variation Among Accessions

George F. Antonious,[1] Tejinder S. Kochhar,[2] Robert L. Jarret,[3] and John C. Snyder[4]

[1]Department of Plant and Soil Science, Kentucky State University, Frankfort, KY
[2]Department of Biology, Kentucky State University, Frankfort, KY
[3]U.S. Department of Agriculture/Agriculture Research & Service, USDA/ARS Plant Genetic Resources Conservation Unit, Griffin, GA
[4]Department of Horticulture, University of Kentucky, Lexington, KY, USA

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The U.S. Department of Agriculture (USDA) pepper (*Capsicum* spp.) germplasm collection contains several thousand members or accessions. Many of these species and cultivars have not been analyzed for their concentrations of ascorbic acid, capsaicin, and total phenolic compounds, which are important antioxidants having a number of benefits for human health. The objective of this investigation was to select candidate accessions of hot pepper having high concentrations of ascorbic acid, capsaicin, free sugars, and total phenols for use as parents in breeding for these compounds. Seventeen accessions of pepper from the core *Capsicum* germplasm collection (four accessions of *Capsicum chinense*; five accessions of *C. baccatum*; six accessions of *C. annuum*; and two of *C. frutescens*) were field grown and their mature fruits were analyzed for their antioxidant composition. Concentrations of these compounds tended to be higher in *C. chinense* and *C. baccatum*, than in *C. annuum* and *C. frutescens*. Across all accessions the concentration of total phenols was correlated with ascorbic acid ($r = 0.97$) and free sugars ($r = 0.80$). Concentrations of total phenols (1.4, 1.3, and 1.3 mg g^{-1} fruit) and ascorbic acid (1.6, 1.2, and 1.3 mg g^{-1} fruit) were significantly greater in PI-633757, PI-387833, and PI-633754, respectively, compared to other accessions analyzed. Total capsaicinoids concentrations were greatest (1.3 mg g^{-1} fruit) in PI-438622 and lowest (0.002 mg g^{-1} fruit) in Grif-9320. The great variability within and among *Capsicum* species for these phytochemicals suggests that these selected accessions may be useful as parents in hybridization programs to produce fruits with value-added traits.

Key Words: *Capsicum* spp.; Phytochemicals; Ascorbic acid; Total phenols; Free sugars; Capsaicin.

Received February 3, 2006.
Address correspondence to G. F. Antonious,Department of Plant and Soil Science,Water Quality/ Environmental Toxicology, Land Grant Program, 218 Atwood Research Center, 400 East Main Street, Kentucky State University, Frankfort, KY, 40601, USA; Phone: 502/597-6005; Fax: 502/597-6381; E-mail: george.antonious@kysu.edu

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INTRODUCTION

There is a growing interest in the enhancement of compounds in food having health-promoting attributes such as antioxidant properties, and which were previously regarded as non-nutritive.[1] Plants contain numerous non-nutritive bioactive compounds known as "phytochemicals." Many of these components, including phenolic compounds, are antioxidants in nature.[2] Phenolic compounds are the largest category of phytochemicals and the most widely distributed in the plant kingdom.[3] Plant phenolics include simple phenols, flavonoids, anthocyanins, lignans and lignins, stilbenes and tannins. Phenols are often associated with plant defense mechanisms against predators, bacteria and fungi.[4] Resistance of certain host plants may depend partially or completely on their phenolic compounds.[5–7] The role of phenols as antioxidants in food with properties similar to vitamins C, E, and ß-carotene have prompted a number of studies of these compounds.[8] By virtue of their antioxidant activity, they may play a role in the protection of cardiovascular health and prevention of certain cancers.[9]

Capsaicin, the pungent principle in fruit of members of the genus *Capsicum*, also exhibits antioxygenic activity[10] and potent antimutagenic and anticarcinogenic properties.[11]

The USDA *Capsicum* germplasm collection contains many thousands of accessions of the genus *Capsicum*, however limited information is currently available on their composition.[12,13] Variability in the presence and concentration(s) of phytochemicals in pepper species can be a factor affecting the selection of pepper for breeding programs. The objectives of this investigation were to determine the concentrations of phenols, ascorbic acid, and capsaicin in seventeen cultivated hot pepper accessions, and to select candidate accessions of hot pepper having high concentrations of phenolic compounds, ascorbic acid, and capsaicin for use as a source of antioxidants or as parents in hybridizations in the USDA breeding programs.

MATERIALS AND METHODS

Seeds of 17 hot pepper accessions were established in the greenhouse in the spring of 2004 and transplanted to the field at the Georgia Experiment Station (Griffin, GA) in June in a sandy-loam soil of 1.3% organic matter. Four accessions of *Capsicum chinense* (PI-387833, PI-387836, PI-438622, and PI-585253), five accessions of *C. baccatum* (PI-633754, PI-633755, PI-633756, PI-633757, PI-633834), six accessions of *C. annuum* (PI-414729, PI-419133, PI-430490, Grif-14486, Grif-14487, and Grif-14513), and two accessions of *C. frutescens* (PI-387834 and Grif-9320) were harvested and transported overnight to Kentucky State University, Frankfort, KY for fruit analysis. Mature fruits of

the seventeen *Capsicum* accessions were analyzed for total phenols, ascorbic acid, free (soluble) sugars, and the two principal capsaicinoids (capsaicin and dihydrocapsaicin). Accessions were selected to represent cultivated species, a wide gene base, and the geographic origin of the accessions.[14]

Representative fruit samples (20 g) were blended with 150 mL of ethanol to extract phenols. Homogenates were filtered through Whatman No. 1 filter paper and 1 mL aliquots of filtrate were used for determination of total phenols[15] against a standard calibration curve (1 to 16 μg mL^{-1}) of chlorogenic acid (Fisher Scientific Company, Pittsburgh, PA, USA). Ascorbic acid was extracted by blending 20 g of fruit with 100 mL of 0.4% (w/v) oxalic acid solution[15] and determined by the 2,6-dichlorophenolindophenol method.[16] Reducing (soluble) sugars in 25 g of fruits were extracted with 80% ethanol and quantified by the method described by VanEtten et al.[17]

Capsaicin was extracted by steeping 5–10 fresh fruits in 120 mL of methanol and subsequently blending at a high speed for one min. The solvent extracts were decanted through 55 mm Whatman 934-AH glass microfiber filter discs (Fisher Scientific, Pittsburgh, PA) using vacuum filtration. Aliquots of 10 mL of the extract were passed through 0.45 μm GD/X disposable syringe filter (Fisher Scientific, Pittsburgh, PA) and 1 μL was injected into a Hewlett-Packard (HP) gas chromatograph (GC) equipped with a nitrogen-phosphorus detector (NPD). GC quantification was accomplished using a 25 m $\times$ 0.20 mm internal diameter (ID) capillary column with 0.33 μm film thickness (HP-1). Operating conditions were 230, 250, and 280° for injector, oven, and detector, respectively. Carrier gas (He) flow rate was 5.2 mL min^{-1}. Quantification was based on average peak areas of 1 μL injections from external standard solutions of capsaicin and dihydrocapsaicin prepared in methanol. Under these conditions, retention times (R_t) were 11.40 and 11.75 min, for capsaicin and dihydrocapsaicin, respectively. A HP gas chromatograph (GC) model 5890A equipped with a mass chromatograph (GC/MS) operated in total ion monitoring was also used for identification and confirmation of individual peaks. Mass spectrometry indicated two molecular ions at m/z 305 and m/z 307 which corresponded to capsaicin and dihydrocapsaicin, respectively. Purified standards of capsaicin and dihydrocapsaicin were obtained from Sigma-Aldrich Inc. (Saint Louis, MO 63103, USA) and used to prepare calibration curves. To determine the performance of the analytical procedures, capsaicin and dihydrocapsaicin (100 μg g^{-1} fresh fruit) were added to 25 g of accession Grif-9320. Recoveries of the added capsaicin and dihydrocapsaicin were 98% and 95%, respectively. Recoveries of added chlorogenic acid, ascorbic acid, and sugars to fruits of accession Grif-9320 (*C. frutescens*) were 93, 90, and 88%, respectively. Concentrations of total phenols, ascorbic acid, capsaicinoids, and free sugars in hot pepper fruits were analyzed using analysis of variables analysis of variance (ANOVA) procedure. Means were compared using Duncan-Waller LSD test.[18]

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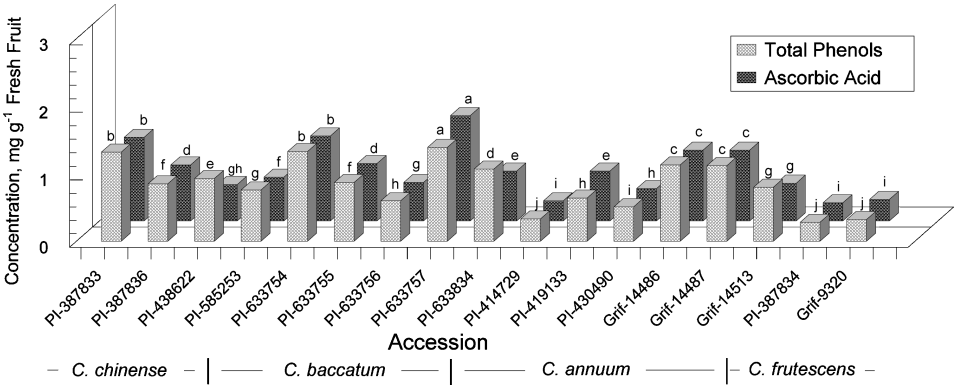

Figure 1: Concentrations of total phenols and ascorbic acid in the fruits of seventeen accessions of four *Capsicum* species. Bars accompanied by different letter(s) for each compound indicate significant differences ($P < 0.05$) between accessions using Duncan's least significant difference (LSD) test.

RESULTS AND DISCUSSION

Concentrations of total phenols (1.4, 1.3, and 1.3 mg g^{-1} fresh fruit) and ascorbic acid (1.6, 1.2, and 1.3 mg g^{-1} fresh fruit) were significantly greater in accessions PI-633757, PI-387833, and PI-633754, respectively, compared to other accessions analyzed (Fig. 1). These accessions may be useful as parents in breeding programs to produce the high phenol and ascorbic acid containing varieties. On the contrary, PI-414729, PI-387834, and Grif-9320 had the lowest contents of both phenols and ascorbic acid (Fig. 1), while PI-438622 had the lowest sugar content (Fig. 2). Pronounced differences in total capsaicinoids

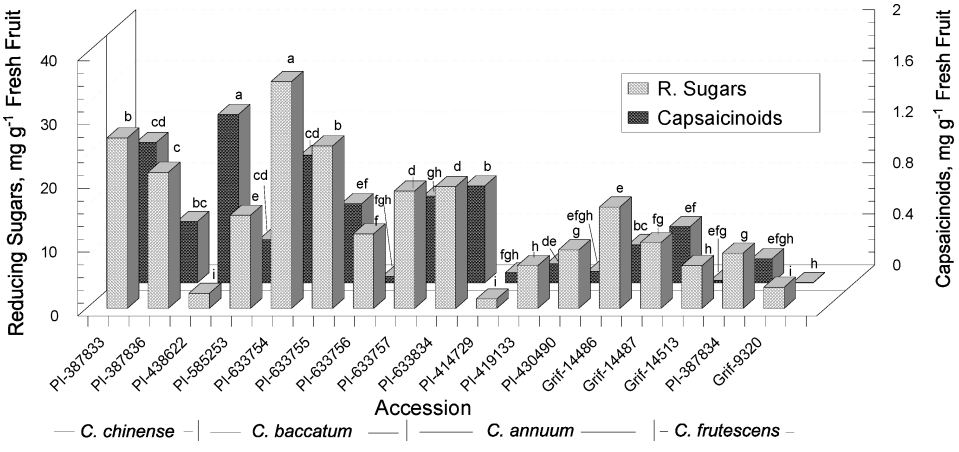

Figure 2: Concentrations of reducing (soluble) sugars and capsaicinoids (capsaicin plus dihydrocapsaicin) in the fruits of seventeen accessions of four *Capsicum* species. Bars accompanied by different letter(s) for each compound indicate significant differences ($P < 0.05$) between accessions using Duncan's LSD test.

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Table 1: Concentrations[†] of total phenols, ascorbic acid, capsaicin, and reducing sugars in the fruits of several accessions of *Capsicum* species grown under field conditions (University of Georgia U.S. Department of Agriculture/Agricultural Research Service, USDA/ARS, Griffin, GA).

Capsicum species/ Accession	Concentration, mg fruit^{-1}				
	Total phenols	Ascorbic acid	Capsaicinoids	Reducing sugars	Wt. (g) of each fruit[‡]
C. chinense					
PI-387833	2.7 h	2.50 ij	2.2 cd	53.5 hg	2.0 p
PI-387836	4.6 f	4.4 e	2.6 bc	113.9 e	5.3 k
PI-438622	8.0 d	4.6 e	10.6 a	20.2 j	8.5 h
PI-585253	7.3 e	6.2 d	2.2 cd	140.1 c	9.6 g
C. baccatum					
PI-633754	2.8 h	2.7 hij	2.1 cd	75.9 f	2.1 o
PI-633755	2.0 i	2.0 jk	1.4 ef	59.1 g	2.3 n
PI-633756	11.9 b	11.3 b	0.9 fgh	230.5 b	19.8 b
PI-633757	1.3 j	1.5 k	0.7 gh	17.1 j	0.9 q
PI-633834	4.2 fg	2.9 ghi	2.9 b	13.7 f	3.9 l
C. annuum					
PI-414729	3.7 g	3.3 fgh	0.9 fgh	16.6 j	11.2 e
PI-419133	7.1 e	8.1 c	1.6 de	73.9 f	11.0 f
PI-430490	7.0 e	6.5 d	1.2 efgh	123.7 de	13.5 c
Grif-14486	9.2 c	8.6 c	2.5 bc	129.8 cd	8.2 i
Grif-14487	3.8 g	3.6 fg	1.5 ef	35.2 i	3.4 m
Grif-14513	30.0 a	21.1 a	1.2 efg	251.5 a	37.6 a
C. frutescens					
PI-387834	1.7 ij	1.6 k	1.2 efgh	53.0 gh	6.1 j
Grif-9320	4.1 fg	4.1 ef	0.6 h	41.9 hi	12.7 d

[†]Detectability limits (minimum detectable concentration in μg divided by sample weight in g) for capsaicin and dihydrocapsaicin were similar (0.001 μg g^{-1} fresh fruit).
[‡]Average weight of each fresh pepper fruit (n = 10). Values within a column having different letter (s) are significantly different ($P < 0.05$), using Duncan-Waller LSD test (SAS Institute, 2001).

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(capsaicin plus dihydrocapsaicin) concentrations were found among accessions. Total capsaicin and dihydrocapsaicin concentrations were greatest (1.3 mg g^{-1} fresh fruit) in PI-438622 and lowest (0.002 mg g^{-1} fresh fruit) in Grif-9320. Table 1 shows the concentrations of total phenols, ascorbic acid, capsaicinoids, and reducing sugars per whole fruit. Accession Grif-14513 had the greatest fruit weight (37.6 g) and greatest concentration of total phenols, ascorbic acid, and reducing sugars. One fruit of this accession contained 1.2 mg of total capsaicinoids. The greatest concentration of capsaicinoids (10.64 mg Fruit^{-1}) was found in accession PI-438622 of *C. chinense*.

Strong correlations were observed between total phenols and ascorbic acid and between total phenols and reducing sugars (Table 2), while total capsaicinoids were not correlated with fruit weight or any of the other fruit components analyzed. Our data suggest that great variability exists within and among *Capsicum* species in phytochemicals with antioxidant properties,

Table 2: Pearson's correlation coefficients and probability of significance[†] between pepper fruit components.

	Total phenols	Ascorbic acid	Capsaicinoids[‡]	Reducing sugars
Ascorbic acid	0.97* (<0.0001)[†]			
Capsaicinoids	0.03[ns] (0.92)[†]	−0.08[ns] (0.7561)[†]		
Reducing sugars	0.80* (0.0001)[†]	0.86* (<0.0001)[†]	−0.23[ns] (0.3780)[†]	
Fruit weight	0.93* (<0.0001)[†]	0.93* (<0.0001)[†]	−0.12[ns] (0.6384)[†]	0.76* (0.0003)[†]

Pearson's correlation analysis, (*) indicates significant correlation, ns = not significant ($P > 0.05$).
[‡]Capsaicinoids refer to capsaicin plus dihydrocapsaicin.

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and suggest that these traits might be manipulated via plant breeding or other research approaches to produce fruit with value-added traits.

No single accession contained the highest concentrations of the three antioxidants investigated in this study. PI-633757 (*C. baccatum*) had the highest concentrations of ascorbic acid and total phenols (1.6 and 1.4 mg g^{-1} fresh fruits, respectively) while, PI-438622 (*C. chinense*) had the highest concentration of capsaicinoids (1.3 mg g^{-1} fresh fruits) among the accessions selected in this study. These two accessions (PI-633757 and PI-438622) can be recommended for human consumption as a source of phytochemicals having antioxidant activity.

ACKNOWLEDGMENTS

We thank Zachary Ray and Daddy Boateng for their assistance in preparing hot pepper extracts. This investigation was supported by a grant from US. Department of Agriculture USDA/Cooperative Store Research, Extension, and Education Service (CSREES) to Kentucky State University under agreement No. KYX-2004-15102.

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